Adhesion Molecules on the Plasma Membrane of Epidermal Cells. I. Human Resting Langerhans Cells Express Two Members of the Adherence-promoting CD11/CD18 Family, Namely, H-Mac-1 (CD11b/CD18) and gp 150,95 (CD11c/CD18)

Giuseppe De Panfilis, M.D., Davide Soligo, M.D., Gian Carlo Manara, M.D., Corrado Ferrari, M.D., and Claudio Torresani, M.D.

Department of Dermatology, University of Parma (GDP, GCM, CT); Institute of Medical Sciences, University of Milan (DS); and Department of Histology, University of Parma (CF), Parma, Italy

The CD11/CD18 family of leukocyte adhesion-promoting proteins is comprised of three members, each composed of a shared beta subunit (CD18) noncovalently associated with unique alpha subunits (CD11a, CD11b and CD11c respectively). Such three heterodimers, named LFA-1 (CD11a/CD18), H-Mac-1 (CD11b/CD18) and gp150,95 (CD11c/CD18), are involved in mediating leukocyte adhesion in virtually all phases of the immune responses. Since Langerhans cells are regarded as cutaneous leukocytes, we investigated the expression of the members of the CD11/CD18 family on Langerhans cells.

A vast series of immunostaining procedures was carried out, using monoclonal antibodies anti-CD11a, -CD11b, -CD11c, and -CD18. Normal skin frozen sections and epidermal sheets were investigated by immunohistology and immunofluorescence; suspended freshly isolated epidermal cells were processed using immunogold techniques, performed in both transmission and scanning electron microscopy, including double labeling procedures and semiquantitative analysis of the labeled cells. The results demonstrated the expression on the membrane of Langerhans cells of the CD11b, CD11c and CD18 antigens, thus indicating that at least both the H-Mac-1 (CD11b/CD18) and the gp 150,95 (CD11c/CD18) members of the CD11/CD18 family are detectable on the cell surface of human resting Langerhans cells. Since both such moieties serve as adhesion molecules in (a) cell-cell interactions and in (b) leukocyte migration and localization, the present results suggest that H-Mac-1 and gp 150,95 might display a key role (a) in promoting interactions between Langerhans cells and other cells, and (b) in guiding the migration and localization of Langerhans cells. *J Invest Dermatol 93:60–69, 1989.*

This study was supported by grants from CNR (88.00488.04) and from MPI (40%), Roma, Italy.

This work was presented in part on the occasion of the following Meetings: 17th Annual Meeting of the European Society for Dermatological Research, Amsterdam, The Netherlands, March 29–April 1, 1987 (CD11b expression by Langerhans cells); 2nd International Workshop on Langerhans cells, Lyon, France, April 21–22, 1988 (CD11c expression by Langerhans cells); 15th Annual Meeting of Society for Cutaneous Ultrastructure Research, Nice, France, May 20–21, 1988 (CD18 expression by Langerhans cells); 18th Annual Meeting of the European Society for Dermatological Research, Münich, West Germany, June 19–22, 1988 (quantitative analysis of the CD11/CD18 expression by Langerhans cells).

Reprint requests to: G. De Panfilis, M.D., Clinica Dermatologica Universita', via Gramsci 14, I–43100 Parma, Italy.

Abbreviations:

BSA: bovine serum albumin
CD: cluster of differentiation
EC: epidermal cell(s)
FCS: heat-inactivated fetal calf serum
GAM-G15: 15-nm sized colloidal gold-conjugated goat antimouse IgG antibody
GAM-G40: 40-nm sized colloidal gold-conjugated goat antimouse IgG antibody
gp 150,95: glycoprotein 150,95
HBSS: Hanks's balanced salt solution
H-Mac-1: human Mac-1
IEM: immunoelectronmicroscopy
IF: immunofluorescence
IG: immunogold
IP: immunoperoxidase
LC: Langerhans cell(s)
LFA-1: lymphocyte function-associated antigen 1
LM: light microscopy
moAb: monoclonal antibody(ies)
PBMC: peripheral blood mononuclear cells
PBS: phosphate-buffered saline
RPMI-FCS: RPMI-1640 medium with 10% FCS
SEM: scanning electron microscopy
TEM: transmission electron microscopy

0022-202X/89/$03.50

Several lines of evidence recently indicated that cell adhesion, cell migration, and lymphocyte help and killing all involve related cell surface receptors. Indeed, three receptor families, namely, (1) the VLA, fibronectin receptor family; (2) the gp IIb/IIIa, vitronectin receptor family; and (3) the CD11/CD18 family, are conserved subgroups in a superfamily of adhesive receptors called "integrins" [1,2]. The members of the first and second integrin families are consistently extracellular matrix receptors [1,3].

The third integrin family (CD11/CD18 family) is involved in cellular adhesion in virtually all phases of the immune responses [4,5], and consists of three non-covalently associated alpha/beta heterodimers, which share an identical beta subunit, and are distinguished by three different alpha subunits [6]. In fact, the first heterodimer, called lymphocyte function-associated antigen 1 (LFA-1), has an alpha subunit (CD11a) of 180kD (alpha L) [5–15]; the second heterodimer, called human-Mac-1 (H-Mac-1), has an alpha subunit (CD11b) of 165 kD (alpha M) [6,7,16–20]; the third heterodimer, called glycoprotein 150,95 (gp 150,95), has an alpha subunit (CD11c) of 150 kD (alpha X) [6,7,12,17,21–24]. The three heterodimers share an identical beta subunit (CD18) of 95 Kd [6,7,12,25–28] (Table I). It is now clear that the CD11a-b-c subunits are structurally related transmembrane polypeptides with long extracellular domains including divalent cation-binding sequences; the CD18 subunit is an integral membrane polypeptide whose extracellular portion contains four cysteine-rich homologous repeats.

The three members of the CD11/CD18 family are involved in leukocyte adhesive interactions. In fact, the first member, i.e., LFA-1 (CD11a/CD18), is expressed on most leukocytes and especially on lymphocytes and functions particularly in lymphocyte help, killing and adhesion [5–15,25–28]; the second member, i.e., H-Mac-1 (CD11b/CD18), and the third member, i.e., gp 150,95 (CD11c/CD18), are both expressed on leukocytes generally other than lymphocytes, and especially on monocytes, tissue macrophages and neutrophils, and function as adhesion molecules in cell-cell and cell-substrate interactions [6,7,12,16–28].

Langerhans cells (LC) are fascinating, bone-marrow-derived [29], dendritic epidermal cells which play a key role in the immunological functions of the skin; they can be considered epidermal leukocytes [30,31]. We therefore intended to examine whether LC could express, as well as other circulating and tissue leukocytes do, adhesive molecules on their plasma membrane.

By means of an exhaustive series of immunostaining procedures, we demonstrated that human resting LC clearly express at least both the second (CD11b/CD18) and the third (CD11c/CD18) members of the CD11/CD18 family, and propose the hypothesis that such expressions could possibly be related to LC adherence to other cell types and to LC migration and localization.

MATERIALS AND METHODS

Normal Human Skin Two types of skin samples were utilized, namely skin biopsies and skin slices. Skin biopsies were removed from clinically normal-appearing skin of ten healthy volunteers whose age ranged from 17 to 61 years. The specimens were obtained from either sun-protected or sun-exposed areas. Skin slices were obtained from clinically normal-appearing skin of ten healthy subjects whose age ranged from 21 to 58 years, removed from either breast or abdomen areas by plastic-surgery reduction operations.

Immunoreagents

Primary (Monoclonal) Antibodies: The anti-CD11a, -CD11b, -CD11c, and -CD18 monoclonal antibodies (moAb) used in this study are listed in Table I. In the double immunogold (IG) labeling experiments, a further moAb was used, namely the 5-nm sized colloidal gold-conjugated anti-HLA-DR moAb (E.Y. Laboratories, Inc., San Mateo, CA).

Secondary Antibodies: The following secondary sera and antibodies were used: (1) peroxidase-conjugated goat antimouse Ig serum (Dakopatts, Copenhagen, Denmark), for immunoperoxidase (IP) staining in light microscopy (LM); (2) fluorescein isothiocyanate-conjugated $F(ab')_2$ goat antimouse Ig antibody (Tago, Inc., Burlingame, CA), for immunofluorescence (IF); (3) 15-nm sized colloidal gold-conjugated goat antimouse IgG antibody (GAM-G15, Janssen Pharmaceutica, Beerse, Belgium), for IG-immunoelectronmicroscopy (IEM) labeling in transmission electron microscopy (TEM); (4) 40-nm sized colloidal gold-conjugated goat antimouse IgG antibody (GAM-G40, Janssen), for IG-IEM labeling in scanning electron microscopy (SEM).

Control Reagents: Control immunoreagents included: (1) purified mouse unreactive IgG fraction of appropriate isotype (Chemicon International Inc., El Segundo, CA); (2) purified unlabeled goat antimouse IgG antibody (Chemicon); (3) unconjugated colloidal gold particles, either 40-nm, 15-nm or 5-nm sized, respectively (Ortho Pharmaceutical Co., Raritan, NJ); (4) purified unlabeled anti-HLA-DR moAb (Becton-Dickinson Monoclonal Center, Mountain View, CA); (5) purified anti-Leu6 (CD1a) moAb (Becton-Dickinson).

Processing of Skin Biopsies Fresh biopsy samples were divided in two parts. The first portion was immediately frozen in liquid

Table I. Distribution on Leukocytes of the Three Members of the Adhesion-promoting CD11/CD18 Family, Namely LFA-1 (CD11a/CD18), H-Mac-1 (CD11b/CD18) and gp 150,95 (CD11c/CD18), and Monoclonal Antibodies Used in This Study to Identify the Subunits of the CD11/CD18 Family

Cluster of Differentiation	Subunit	kD	Cellular Reactivity	moAb Used in This Study
CD11a	alpha L chain	180	T and B lymphocytes (a), most other leukocytes	TS1/22.1.1.17 (b) MHM24 (c)
CD11b	alpha M chain	165	monocytes, macrophages, NK cells, granulocytes, subset of T8 lymphocytes	Anti-Leu-15 (d) 60.1 (e) OKM1 (f) Anti-C3bi (g)
CD11c	alpha X chain	150	monocytes, macrophages (a), NK cells, granulocytes, 2% lymphocytes	Anti-Leu-M5 (d)
CD18	common beta chain	95	most leukocytes	60.3 (h)

(a) Cells expressing large amounts of antigen.
(b) Code number 720, from the third international Workshop on human leukocyte differentiation antigens (Oxford, 1986).
(c) Code number 709, from the third international Workshop on human leukocyte differentiation antigens (Oxford, 1986).
(d) Becton Dickinson Monoclonal Center, Mountain View, CA, USA.
(e) Code number 645, from the third international Workshop on human leukocyte differentiation antigens (Oxford, 1986).
(f) Ortho Pharmaceutical Co, Raritan, NJ, USA.
(g) Dakopatts, Copenhagen, Denmark.
(h) Code number 706, from the third international Workshop on human leukocyte differentiation antigens (Oxford, 1986).

nitrogen, then sectioned 4–5-μm thick by a cryostat, and prepared for the in situ IP studies in LM. The second portion was trimmed, split-cut and floated on a 0.25% solution of trypsin (Gibco, Paisley, U.K.) in Hanks' balanced salt solution (HBSS, Gibco), 45 min at 37°C, and the epidermal sheets were prepared for the IF studies.

Immunoenzymatic Studies on Vertical Sections After blocking the endogenous peroxidases, a double-layer procedure was performed on the cryostat sections, using in the first step the moAb of the CD11/CD18 panel (and on serial sections, as control, the anti-CD1a moAb) usually diluted 1:40 in PBS for 45 min at room temperature, and, in the second step, the peroxidase-conjugated secondary antibody diluted 1:10 in PBS, for 60 min at room temperature. The diaminobenzidine reaction then revealed the peroxidase activity. The immunohistologic method used is extensively described elsewhere [32].

Immunofluorescence Studies on Epidermal Sheets The IF staining was carried out slightly modifying the method previously described [33]. Briefly, epidermal sheets, after extensive washes in phosphate-buffered saline (PBS), were incubated, for the indirect immunostaining, with (first layer) the anti-CD11/CD18 series of moAb (or the control anti-CD1a moAb on separate sheets), diluted 1:40 in PBS, for 6 h, followed by (second layer) the fluorescein isothiocyanate-conjugated secondary antibody, diluted 1:10 in PBS, for 12 h. After washes in PBS and mounting in PBS/glycerol, the sheets were examined under a Zeiss fluorescence microscope in epi-illumination.

Processing of Skin Slices Skin removed by plastic surgery was trimmed of fat and thereafter split-cut by a keratotome, set at 0.4 mm. The resulting fresh slices were removed and immediately utilized for the preparation of the epidermal cell (EC) suspension.

Epidermal Cell Suspension and Langerhans Cell Enrichment The slices were floated, dermal side down, on 0.25% trypsin in HBSS, for 45 min at 37°C, thus allowing the separation of the epidermis from the dermis. The resulting epidermal sheets were treated according to a method described elsewhere [34]. Briefly, the sheets were pooled, shaken and pipetted in HBSS supplemented with 10% heat-inactivated fetal-calf serum (FCS, Gibco). The resulting suspended cells were filtered, sedimented by centrifugation, resuspended, and washed in RPMI-1640 medium with 10% FCS (RPMI-FCS).

For LC enrichment, EC dispersed in RPMI-FCS were layered on the Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient, and subsequently centrifuged for 10 min at 400 *g*. EC at the interface were thereafter collected and resuspended in RPMI-FCS. The viability, assessed by eosin exclusion, was higher than 90%. Such LC-enriched EC suspensions were prepared for the IG-IEM procedures, either for TEM or for SEM.

IMMUNOGOLD LABELING PROCEDURE FOR TRANSMISSION ELECTRON MICROSCOPY

A previously described procedure, with slight modifications, was used [35,36] for the IG-TEM staining. Briefly, LC-enriched EC (5×10^6/ml), after prefixation with 0.1% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 for 10 min, and quenching aldehyde groups by 50 mM glycine, were washed in PBS-0.1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO) pH 7.2, and subsequently subjected to a blocking pretreatment including PBS-1% BSA, 0.2% sodium azide, and 10% decomplemented human AB serum for 30 min. After washings in PBS-0.1% BSA pH 7.2, the moAb were applied to specimen cells, diluted 1:40 in PBS with 1% BSA pH 7.2, 30 min at 4 C. After washes in PBS with 0.1% BSA pH 7.2, the GAM-G15 secondary antibody was used, diluted 1:10 in 1% BSA buffer (0.05 M Tris saline buffer containing 1% BSA and 0.02 M sodium azide) pH 8.2, for 60 min.

After washings in 0.1% BSA buffer pH 8.2, EC specimens were divided into two parts: one of them was directly prepared for the TEM ultrastructural studies, the other one was introduced to the double IG labeling procedure.

Double Immunogold Labeling Procedure EC, stained by (first step) moAb and (second step) GAM-G15 according to the above-mentioned single IG labeling procedure, were subjected to a (third step) blocking incubation with a mouse unreactive IgG fraction (Pel-Freez Biologicals, Rogers, AZ), diluted 1:2 in PBS with 0.1% BSA pH 7.2, for 30 min at 4°C, to block the still-available antigen binding sites of the GAM-G15. Finally, the (fourth step) 5-nm sized gold-conjugated anti-HLA-DR moAb was used, diluted 1:40 in PBS with 1% BSA pH 7.2, 60 min. Such double-labeled EC, after washings in PBS with 0.1% BSA pH 7.2, were then prepared for the TEM ultrastructural studies.

Immunogold Labeling Procedure for Scanning Electron Microscopy The IG labeling for SEM was carried out according to a previously described technique [37,38]. Briefly, the LC-enriched EC were allowed to settle by placing a drop of the cell suspension on glass coverslips, pretreated by 0.1% poly-l-lysine, in a moist chamber, for 30 min at room temperature. After attachment, prefixation with 0.2% glutaraldehyde in PBS pH 7.2 for 5 min, quenching of aldehyde groups by 0.1% glycine in PBS for 10 min, and blocking pretreatment by PBS pH 7.2 containing 1% BSA, 0.2% sodium azide (PBS-BSA buffer) and 10% decomplemented human AB serum, EC were then subjected to an indirect IG procedure, since they were incubated first with the moAb diluted 1:40 in PBS-BSA buffer, 30 min at room temperature, and, after washings in PBS-BSA buffer, with the GAM-G40 secondary antibody, diluted 1:10 in 1% BSA buffer pH 8.2, 60 min at room temperature. After washings in 0.1% BSA buffer, pH 8.2, EC were prepared for SEM ultrastructural studies.

Transmission Ultrastructural Studies The EC suspensions prepared for TEM procedures, both single and double IG labeled, were washed in PBS, pH 7.2, and then fixed with 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Cells were subsequently packaged in 2% Bacto-Agar (Difco Laboratories, Detroit, MI) at 45°C, postfixed in potassium ferricyanide-reduced osmium tetroxide, dehydrated in acetone, and finally embedded in Durcupan ACM (Fluka, Buchs, Switzerland). Ultrathin sections were obtained by a LKB III ultramicrotome and were examined, either post-stained with lead citrate and uranyl acetate or absolutely uncounterstained, at a Philips EM 300 electron microscope. To evaluate the gold labeling at the TEM level, a cell was considered positive when at least three gold particles were observed attached at its plasma membrane per section [39].

Scanning Ultrastuctural Studies The EC prepared for SEM procedures were washed in PBS pH 7.2, postfixed in 2% glutaraldehyde in PBS, dehydrated in graded alcohols, critical-point dried from CO_2, coated with 200 Å of evaporated carbon, and observed at a Philips 505 scanning electron microscope [37,38].

Control Experiments

Controls for Methods Specificity: For the indirect methods, including IP on vertical sections in LM, IF on epidermal sheets, IG single labeling in TEM and IG labeling in SEM, the negative controls consisted of: (1) replacement of the moAb by mouse unreactive IgG of appropriate isotype; (2) replacement of the moAb by buffer solution; and (3) inhibition of staining by preincubation, before the conjugated goat antimouse IgG antibody, with the unlabeled goat antimouse IgG antibody. An additional negative control in the IEM (both in TEM and in SEM) studies was the replacement of the GAM-G15 (TEM) and GAM-G40 (SEM) by unconjugated gold particles of the same size. Controls for the four-step double IG procedure included, together with the above-mentioned controls, inhibition of the 5-nm sized gold staining by preincubation, before the 5-nm sized gold-conjugated anti-HLA-DR moAb, with the unconjugated anti-HLA-DR moAb; and replacement of the 5-nm sized gold-conjugated moAb by unconjugated 5-nm sized gold particles.

Controls for Immunoreagents: Positive controls for moAb were carried out on normal human lymph node, using IF and IP-LM immunostainings.

In addition, peripheral blood mononuclear cells (PBMC) from healthy donors were subjected to the same IG-IEM methods used for EC, although they were slightly modified for PBMC processing according to our previously described procedures, both in TEM [35,36] and in SEM [37], to reveal the ability of the immunoreagents to detect (1) the CD11a- and CD18-positivity of most PBMC, (2) the CD11b- and CD11c-positivity of monocytes, and (3) the CD11b-positivity of a subset of T8 lymphocytes (Table I).

Later, in situ IEM studies were performed on untrypsinized, paraformaldehyde slightly prefixed, normal human-skin frozen sections to confirm the CD11/CD18 results obtained on trypsinized, glutaraldehyde prefixed, suspended EC using a pre-embedding in situ IG method in TEM developed by us [40,41].

A final control, performed in IEM, to confirm the H-Mac-1 and gp 150,95 positivities of LC, was carried out by a blocking experiment incubating LC-enriched EC first with the anti-CD1a moAb (blocking step) and subsequently with the anti-CD11b, -CD11c and -CD18 moAb.

Semi-quantitative Analysis of the Labeling A semi-quantitative evaluation of the number of binding sites at the surface of positive cells [35,42] was performed at the transmission electron microscope, counting the number of gold particles along the circumference of the cell surface of the CD11/CD18-positive LC. In fact, the number of gold granules per section of cell surface was scored, analyzing 40 randomly chosen single-labeled LC sectioned near their midplane [35,42]. Finally, the means and the standard errors were calculated.

RESULTS

Light Microscopy and Fluorescence Immunostainings Immunohistologic studies in skin vertical cryostat sections first revealed that absolutely no dendritic CD11a-positive EC were detectable. On the other hand, the same IP technique resulted only in a faint visualization of both CD11b- and CD18-positive dendritic EC; moreover, serial sections revealed that the CD11b- and CD18-positive EC were less numerous in comparison to the CD1a-positive EC. By contrast, a good visualization of well-reactive CD11c-positive dendritic EC was achieved; further, serial sectioning revealed that the estimated number of the CD1a-positive EC was similar to that of the CD11c-positive EC.

Sheet preparations in IF revealed that practically no dendritic CD11a-positive EC were visible. On the contrary, some dendritic CD11b- and CD18-positive EC were observed, although the staining results were weak. However, a good visualization of CD11c-positive dendritic EC was achieved (Fig 1), and their estimated number was similar to that of the CD1a-positive cells of control sheets.

SINGLE (Figs 2,3,4,5) AND DOUBLE (Fig 6) IMMUNOGOLD STAININGS IN TRANSMISSION ELECTRON MICROSCOPY

Positive cells within the LC-enriched EC suspension were identified, at the TEM level, by the presence of electron-dense gold particles scattered along the circumference of the cell section (Figs 2–4).

Figure 1. Epidermal sheet preparation. Immunofluorescence labeling of CD11c-positive dendritic epidermal cells. Bar = 50 μm.

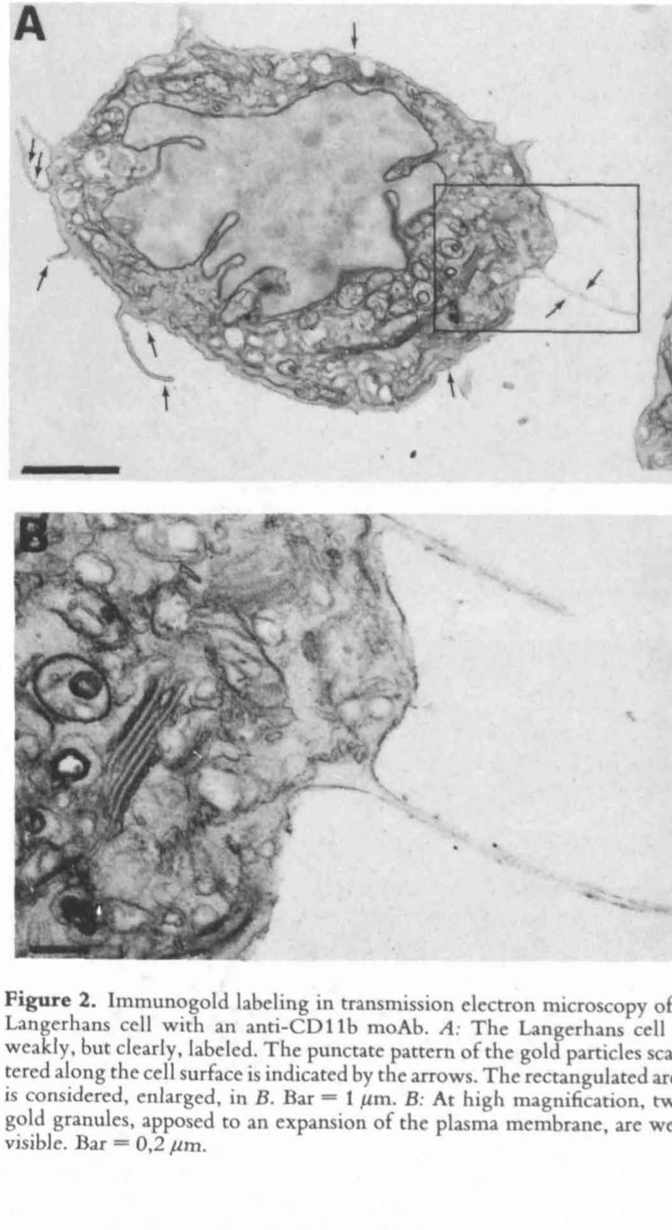


Figure 2. Immunogold labeling in transmission electron microscopy of a Langerhans cell with an anti-CD11b moAb. *A:* The Langerhans cell is weakly, but clearly, labeled. The punctate pattern of the gold particles scattered along the cell surface is indicated by the arrows. The rectangulated area is considered, enlarged, in *B*. Bar = 1 μm. *B:* At high magnification, two gold granules, apposed to an expansion of the plasma membrane, are well visible. Bar = 0,2 μm.

The labeling dealt with a variable number of randomly dispersed and spatially separated gold particles, single or in very small clusters, apposed at the periphery of the positive cells (Figs 2–4), while in the cytoplasm no gold granules were observed (Figs 2–5).

Keratinocytes were virtually CD11a-, CD11b-, CD11c- and CD18-negative. Birbeck granules bearing LC were consistently CD11a-negative. However, all the observed Birbeck granules bearing LC were clearly gold-reactive with both the anti-CD11b (Fig 2) and the anti-CD18 (Fig 4) moAb, and even strongly gold-reactive with the anti-CD11c moAb (Fig 3).

The double IG labeling procedure revealed that all the observed HLA-DR-positive EC (Fig 6) shared the anti-CD11b, -CD11c (Fig 6B), -CD18 (Fig 6C) reactivities, since gold particles of both 5-nm and 15-nm sizes were visible along the surface of such double-labeled EC (Figs 6B, 6C). However, gold particles of 15-nm sizes did not associate with the particles of 5-nm size when the anti-CD11a moAb were used (Fig 6A).

Immunogold Staining in Scanning Electron Microscopy (Fig 7) In the standard secondary electron imaging mode, EC showing the SEM morphology of LC [38] were consistently CD11a-negative, but CD11b- (Fig 7A), CD11c- (Fig 7B) and CD18-positive, as demonstrated by gold particles, as white dots, distributed on the whole cell membrane (Figs 7A, 7B). It was thus

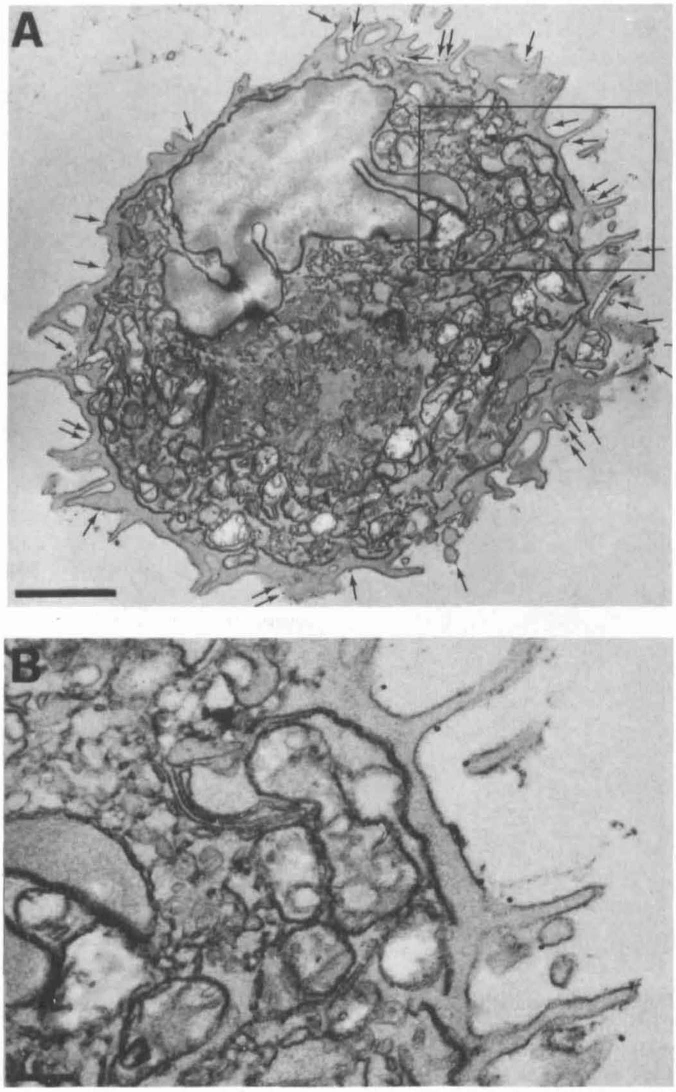


Figure 3. Immunogold labeling in transmission electron microscopy of a Langerhans cell with the anti-CD11c moAb. *A:* The Langerhans cell is strongly labeled. In fact, numerous gold granules *(arrows)* decorate, in a punctate pattern at this low magnification, the cell membrane. The rectangulated area is considered, enlarged, in *B*. Bar = 1 μm. *B:* At high magnification, some gold granules apposed to the cell surface are easily observed. Bar = 0,2 μm.

clear that large numbers of gold granules were visible on the surface of the CD11b- (Fig 7A) and CD18-positive LC, whereas the CD11c-positive LC showed a higher number of gold particles on the cell surface (Fig 7B). The keratinocytes (Fig 7C) were CD11a-, CD11b-, CD11c- and CD18-negative.

Controls Controls for method specificity including immunostaining techniques in LM, IG-TEM procedures (Fig 5), and IG-SEM procedures (Fig 7C) all proved negative.

Controls for immunoreagents were positive. Specifically, anti-CD11a moAb stained, within the PBMC population, lymphocytes and, although to a lesser extent, NK cells and monocytes; anti-CD11b and -CD11c stained monocytes and NK cells; anti-CD11b additionally stained a small subset of lymphocytes, as described [19,20]; anti-CD18 stained most PBMC.

In addition, the in situ IEM studies on normal human skin confirmed the CD11b-, CD11c- and CD18-positivity of unisolated LC, as recently shown by us [41].

Finally, the blocking experiment showed that the anti-CD11b, anti-CD11c and anti-CD18 moAb reacted with LC in a manner strictly similar to that observed in the experiments performed without blocking.

SEMIQUANTITATIVE ESTIMATION OF THE CD11/CD18 EXPRESSION BY LANGERHANS CELLS

The comparative quantification of gold particles fixed on the plasma membrane of LC per cell section (midplane) [35,42] after labeling in TEM with moAb detecting the members of the CD11/CD18 family are shown in Table II. Since, as mentioned above, in TEM a cell is considered positive when at least three gold particles are linked to its section surface [39], it is clear that this semiquantitative analysis demonstrates that LC are virtually CD11a-negative (0.3 ± 0.6 particles per LC section), both CD11b (6.9 ± 10.1 particles per LC section) and CD18 (7.5 ± 4.9 particles per LC section) -positive, and strongly CD11c-positive (35.0 ± 27.7 particles per LC section).

DISCUSSION

The present investigation demonstrates the reactivity of the LC plasma membrane with the anti-CD11b, anti-CD11c, and anti-CD18 moAb, thus confirming our preliminary findings [43-46]. As mentioned above, the CD11b subunit is non-covalently asso-

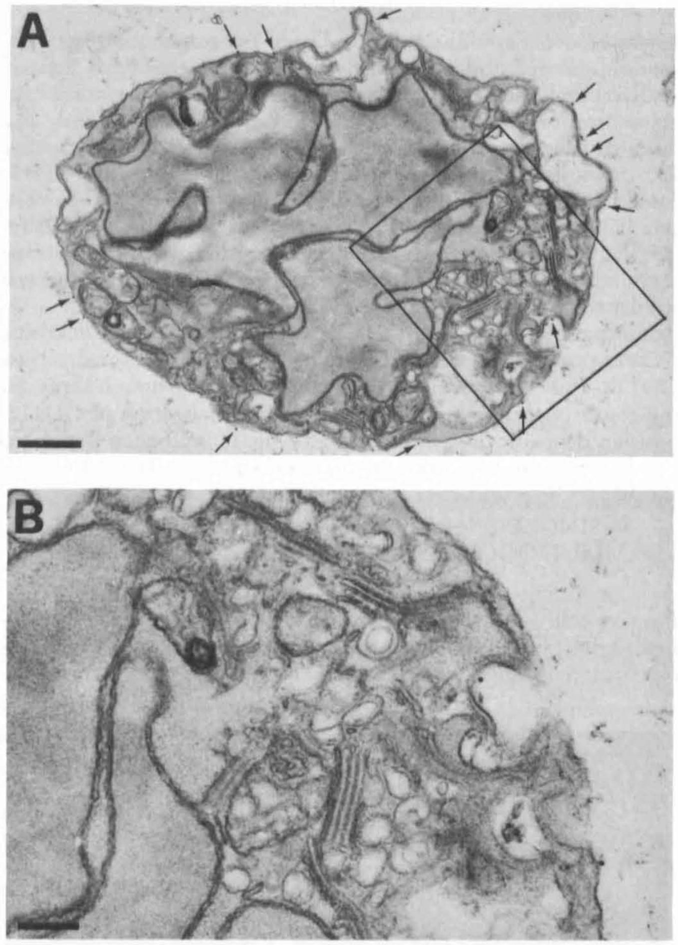


Figure 4. Immunogold labeling in transmission electron microscopy of a Langerhans cell with the anti-CD18 moAb. *A:* The Langerhans cell is weakly, but clearly, labeled. Gold particles *(arrows)* are faintly visible at this magnification, in a punctate pattern. The rectangulated area is considered, enlarged, in *B*. Bar = 0,5 μm. *B:* At high magnification, two gold granules apposed to the cell membrane are well evident. Bar = 0,2 μm.

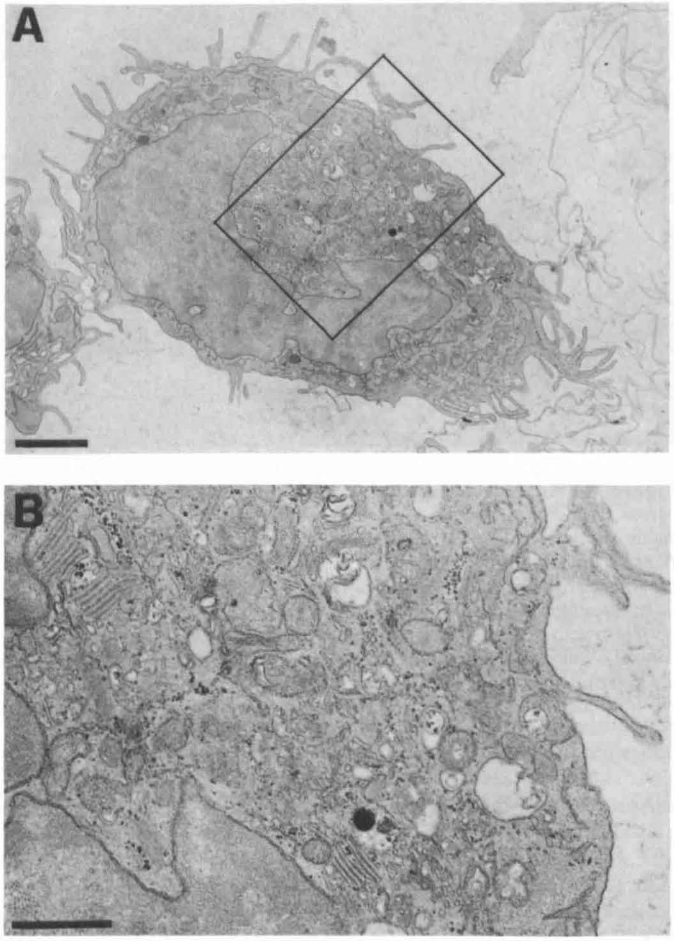


Figure 5. Immunogold labeling in transmission electron microscopy of a Langerhans cell with unreactive mouse IgG of appropriate isotype, instead of moAb (negative control). *A:* The Langerhans cell is unlabeled, since no gold particles are visible along the cell surface. The rectangulated area is considered, enlarged, in *B*. Bar = 1 μm. *B:* Even at high magnification, absolutely no gold particles are observed on the plasma membrane. Bar = 0,5 μm.

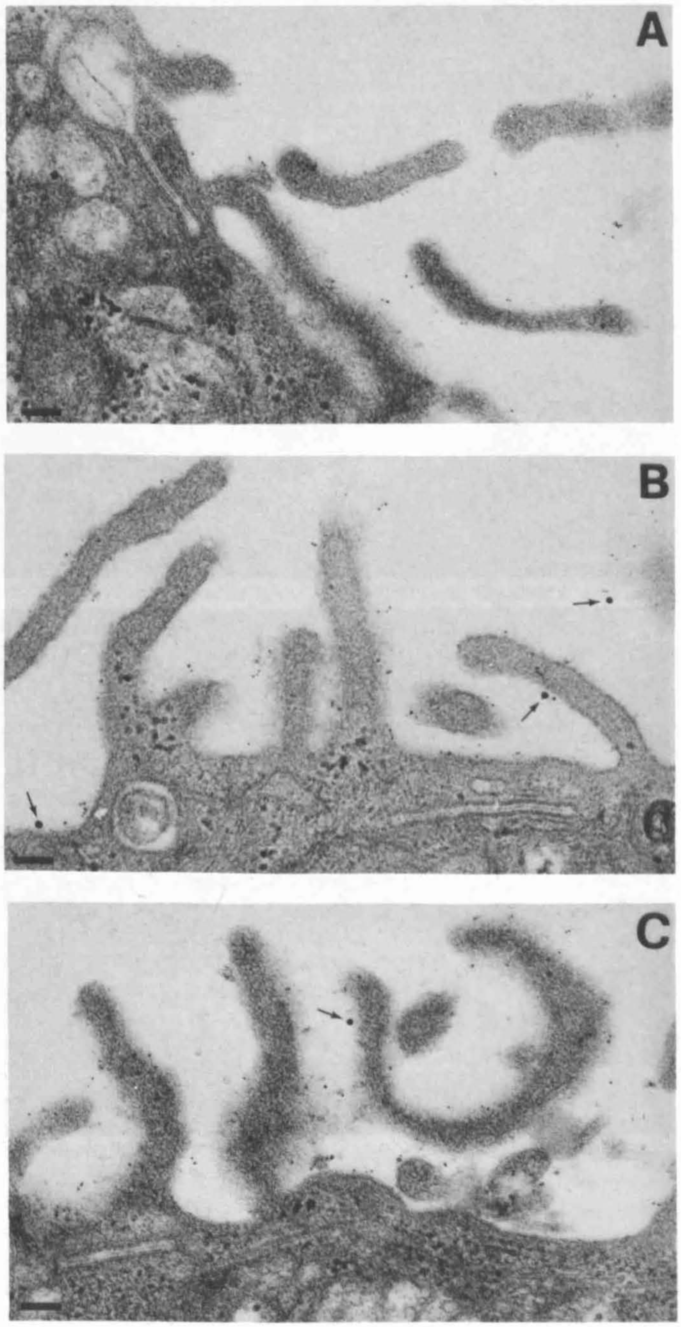
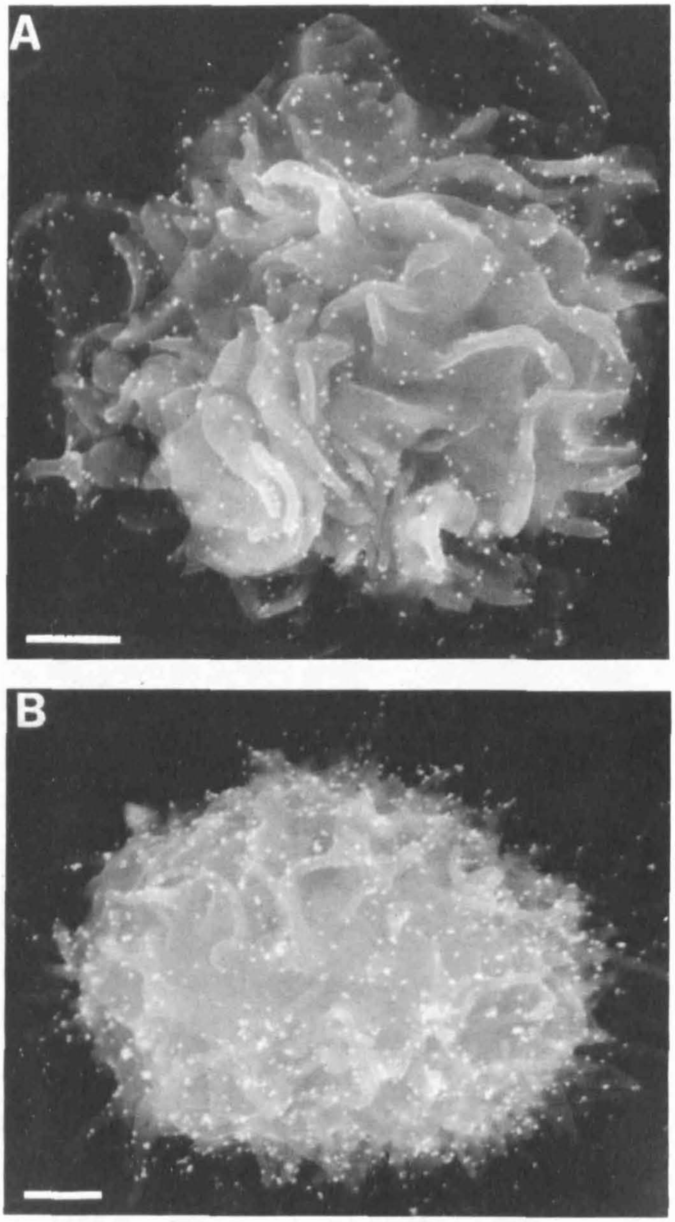


Figure 6. Double immunogold staining in transmission electron microscopy of Langerhans cells with moAb detecting the subunits of the CD11/CD18 family (indirectly labeled by 15-nm sized gold particles) and anti-HLA-DR moAb (directly labeled with 5-nm sized gold particles); poststained sections. *A:* Detail of a Langerhans cell incubated with an anti-CD11a moAb and with anti-HLA-DR moAb. The cell is CD11a-negative, since no gold particles of 15-nm size are visible along the cell surface. By contrast, many 5-nm sized gold particles decorate the membrane, indicating the HLA-DR-positivity. Bar = 0,1 μm. *B:* Detail of a double-labeled Langerhans cell. The CD11c antigens are detected by the 15-nm sized gold particles *(arrows)*, whereas the HLA-DR antigens are detected by the 5-nm sized gold particles. Bar = 0,1 μm. *C:* Detail of a double-labeled Langerhans cell. The 15-nm sized gold particles (only one in this area: *arrow*) indicate the presence of the CD18 antigen, while the numerous 5-nm gold particles indicate the presence of HLA-DR antigens. Bar = 0,1 μm.

ciated with CD18 subunit to form the heterodimer H-Mac-1 (CD11b/CD18) [6]; similarly, the CD11c subunit is non-covalently associated with the CD18 subunit to form the heterodimer gp 150,95 (CD11c/CD18) [6]. On the other hand, the three members (LFA-1, H-Mac-1 and gp 150,95) of the CD11/CD18 family are usually recognized employing the moAb detecting the subunits (CD11a, CD11b, CD11c and CD18) which compose them [7,18,21,24]. In this investigation, therefore, we assess the expression on the membrane of human epidermal resting LC of at least the second (H-Mac-1) and the third (gp 150,95) members of the adhesion-promoting CD11/CD18 family.

In the current study we show that LC do not apparently express the CD11a subunit, since, using two anti-CD11a moAb (TS/22.1.1.17 and MHM24), all the immunostainings including the sensitive [35,36,42] IG-TEM method and even the highly sensitive [37,38] IG-SEM method gave virtually negative results. It seems therefore [7,18,21,24] possible, although within the limits of the immunostaining methods herein used, to conclude that LC plasma membrane does not seemingly express the LFA-1 (CD11a/CD18) glycoprotein, since, though the CD18 subunit is certainly expressed by LC surface [45, present study], LC membrane lacks indeed CD11a. On the other hand, a hypothetical LFA-1 expression by LC, which may be regarded as an epidermal leukocyte [30,31], might, to some extent, have been expected, since the LFA-1 moiety [5], although especially expressed by lymphocytes [8–11,14,15], seems indeed to be present on most leukocytes including



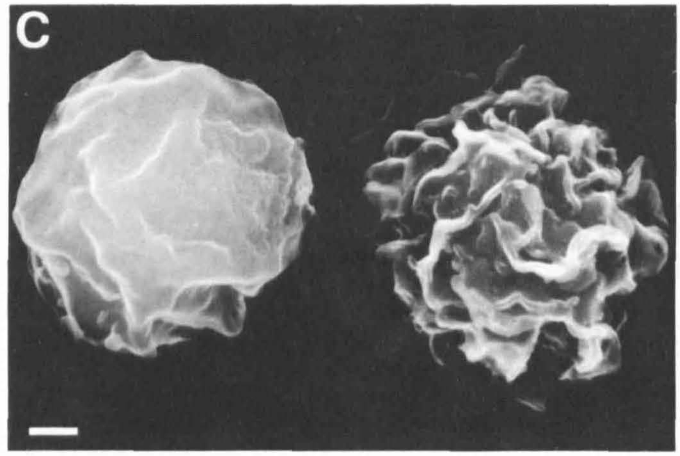


Figure 7. Immunogold labeling in scanning electron microscopy of epidermal cells. *A:* Langerhans cell labeled with an anti-CD11b moAb is appreciable. In fact, gold particles *(white dots)* are visible on the cell surface. Bar = 1 μm. *B:* Numerous gold particles *(white dots)* are visible on the surface of a Langerhans cell labeled with the anti-CD11c moAb. Bar = 1 μm. *C:* Immunogold staining in scanning electron microscopy of epidermal cells with unreactive mouse IgG of appropriate isotype instead of moAb (negative control). The morphological details reveal that the epidermal cell on the left is a keratinocyte, while the epidermal cell on the right is a Langerhans cell. No gold particles are visible on the surface of both epidermal cells. Bar = 1 μm.

monocytes [13]. Certainly, a hypothetical CD11a expression by LC, e.g., possibly detectable by immunoprecipitation studies or using moAb other than those utilized in the present study, cannot be ruled out: in fact, the CD11a epitopes recognized by the moAb used in this investigation might theoretically be either glutaraldehyde and/or trypsin sensitive, or somehow masked, as demonstrated for other epitopes [47]. However, the moAb used here actually succeeded in recognizing the CD11a subunit of CD11a-positive leukocytes including the PBMC of the positive controls performed in the current study. In addition, a mounting body of evidences exists indicating that the most important role of the LFA-1 heterodimer is to strengthen adhesion of lymphocytes (expressing indeed the LFA-1 moiety) to cells bearing specific antigens, perhaps by binding to molecule (presumably the "ICAM-1" molecule) on the antigen-presenting cell or target cell, thereby increasing the range of avidities over which antigen-specific interactions can be effective [48–52]. It seems therefore conceivable that the LFA-1 expression has to be expected on the membrane of lymphocytes interacting with LC rather than on the membrane of the antigen-presenting LC. Instead, LC should express, other than LFA-1, a ligand for the lymphocytic LFA-1; further, such a ligand cannot be the LFA-1 itself, since the possibility of like-like recognition in which LFA-1 on one cell might bind LFA-1 on another cell seems to have been ruled out [5,52]. Therefore, a presence of LFA-1 on the membrane of LC does not seem necessary for the LC antigen presenting function. On the other hand, LC functions, for which adhesive properties are required, can well be subserved by the expression on the LC membrane of the other two members of the adherence-promoting CD11/CD18 family, namely H-Mac-1 and gp 150,95, as shown here.

In this investigation, we extend our previous data [43] suggesting the expression of the CD11b subunit on the membrane of human resting LC. By contrast, the majority of the data reported by several investigators failed to detect anti-CD11b reactive dendritic EC in normal human skin [53–57]. Indeed, such investigations were performed on immunohistological frozen sections [53–57]; thus, it seems likely that the limited amounts of exposed antigenic moieties could escape detection by such conventional staining procedures [58]. In fact, all the observed LC showed their clear, although weak, CD11b-positivity when more-sensitive techniques were used, such as the IG-TEM procedure [35,36,42], and, even to a higher extent, the IG-SEM procedure [37,38]. Further, since LC are the only HLA-DR-positive EC [59,60], the double-staining IG-TEM experiments confirmed that all the HLA-DR-positive EC also were

Table II. Density Per Cell Section (Midplane) of Gold Particles Bound to the Plasma Membrane of Langerhans Cells Labeled with Monoclonal Antibodies Detecting the Subunits of the CD11/CD18 Family (Transmission Electron Microscopy Examination)

moAb	Number of Examined LC Sections	Number of Gold Particles per LC Section (m ± sd)
TS1/22.1.1.17	40	0.4 ± 0.7
MHM24	40	0.2 ± 0.5
Anti-Leu-15	40	5.0 ± 6.5
60.1	40	7.6 ± 4.6
OKM1	40	3.8 ± 3.1
Anti-C3bi	40	11.3 ± 17.5
Anti-Leu-M5	40	35.0 ± 27.7
60.3	40	7.5 ± 4.9
Anti-CD11a	80	0.3 ± 0.6
Anti-CD11b	160	6.9 ± 10.1
Anti-CD11c	40	35.0 ± 27.7
Anti-CD18	40	7.5 ± 4.9

CD11b-positive. Finally, our in situ IEM studies on slightly prefixed skin sections recently confirmed the CD11b staining even of unseparated LC [41].

Here we demonstrate, confirming our preliminary findings [44], that human resting epidermal LC express considerable amounts of CD11c. In fact, not only the highly sensitive IG-IEM methods, but also the IF and LM immunostainings clearly revealed the Leu-M5 (CD11c) positivity of LC. Using a different anti-CD11c moAb, named 3.9, and a conventional indirect IP method in LM, others apparently failed to detect LC in skin sections, but revealed 3.9-positive LC in expanded skin lymphnodes from samples of dermatopathic lymphadenopathy [24]. In agreement with our results, another report [61] claimed the LM immunocytochemical CD11c staining of epidermal dendritic cells, using both the same moAb used by us (SHCL3) and another anti-CD11c moAb, namely KB90. Further, the KB90 reactivity of epidermal dendritic cells was strongly evident, and was even more consistent than the stainings of the same cells obtained using a large panel of moAb detecting other antigens [61]. In addition, the semiquantitative analysis performed here clearly stated that a high number of gold granules decorated the membrane of IG-labeled CD11c-positive LC (Figs 3,6B,7B). Since it is well established that the number of antigenic sites may be, although indirectly, estimated by the densities of gold particles bound to the cell surface [62], it is tempting to conclude that the CD11c expression by LC, revealed by high densities of IG labelings and by even conventional immunocytochemical LM immunostainings, is strong indeed.

Finally, the current study assesses the expression of CD18 by human resting LC. Such an expression, which confirms our preliminary results [45], is not surprising, since virtually all leukocytes are known to certainly bear the CD18 moiety [6,7,12,25–28], and was therefore expected on LC, which are certainly considered leukocytes [30,31].

Functional studies are clearly needed to elucidate the role of the adhesive molecules H-Mac-1 (CD11b/CD18) and gp 150,95 (CD11c/CD18) on the membrane of human resting LC. Such studies, however, were not the purpose of the present investigation. Nevertheless, it is presumable that such molecules could play on the LC surface the same functional roles they are known to play on the membrane of other leukocytes. Indeed, both H-Mac-1 and gp 150,95 have a dual function, as adhesion molecules in (1) cell-cell and (2) cell-substrate interactions. In this respect, it is tempting to speculate that the H-Mac-1 and gp 150,95 expressions by the LC plasma membrane could serve (1) promoting interactions between LC and other cells, and (2) guiding the migration and localization of LC.

In fact, it is now well established that both H-Mac-1 and gp 150,95 are able to mediate intercellular adhesion among leukocytes, and are required for an efficient contact between effector cells and their corresponding targets [12,22,27]. Interestingly, the physical association of dendritic cells and T-lymphocytes, which is required for primary immune responses and precedes and is essential for T-cell activation [63], is inhibited by the anti-CD18 moAb [28]. (The CD18 moiety, as mentioned above, is a part of both H-Mac-1 and gp 150,95 molecules.) Such an observation could suggest a role for adhesive proteins of the CD11/CD18 family even in the antigen-presenting process. On the other hand, the importance of physical conjugates formed in situ between LC and lymphocytes, presumably necessary for the antigen presentation, has been well known for many years [64–66]. It seems therefore tempting to hypothesize that the presence of the adhesion-promoting CD11b/CD18 and CD11c/CD18 receptors on LC could subserve, eventually together with other ligand-receptor interactions, adherence between LC and T-lymphocytes, therefore contributing to an efficient antigen presentation.

A considerable amount of evidence is accumulating that both H-Mac-1 and gp 150,95 subserve leukocyte-substrate adhesion, spreading, directed motility and tissue localization [17,23–26]. H-Mac-1 seems particularly important for the recruitment of monocytic cells to inflammatory stimules [16]. The differentiation of blood monocytes to tissue macrophages results in greatly increased cell surface expression of gp 150,95 [17,23,24], thus regulating the monocyte ability to adhere to endothelial cells, diapedese and localize in various organs as tissue macrophages [17]. On the other hand, LC, derived from bone marrow [29], are able to travel from the blood to their cutaneous residence. Interestingly, a subset of mononuclear cells in peripheral blood of extensively burned subjects, thought to represent LC precursors migrating from their bone-marrow origin to the skin, expressed high amounts of H-Mac-1 [67]. In addition, LC are able to migrate from the epidermis to the dermis and dermal lymphatics during immunological reactions involving the skin [66,68]. It is tempting therefore to speculate that the H-Mac-1 and gp 150,95 moieties expressed on the membrane of LC could guide, as well as demonstrated for monocyte-macrophage migration and localization, the migration and localization of LC.

In conclusion, although functional studies are necessary to clarify the role of the second (H-Mac-1) and the third (gp 150,95) members of the adherence-subserving CD11/CD18 family on the surface of LC, such expressions could promote those LC functions which are related both to LC adherence to other cell types and to LC migration and localization.

The authors thank Dr. A. Boochi, Dr. E. Caleffi, Dr. P. Costa, and Dr. R. Zermani for kindly providing skin slices by plastic-surgery reduction operations. Many thanks are also due to Dr. R. M. Devoto for her technical cooperation. The expert assistance of Dr. A. Zucchi for the statistical analysis is gratefully acknowledged.

REFERENCES

1. Ruoslahti E, Pierschbacher MD: New perspectives in cell adhesion: RGD and Integrins. Science 238:491–497, 1987
2. Hynes RO: Integrins: a family of cell surface receptors. Cell 48:549–554, 1987
3. Hemler ME: Adhesive protein receptors on hematopoietic cells. Immunol Today 9:109–113, 1988
4. Springer TA, Anderson DC: The importance of the Mac-1, LFA-1 glycoprotein family in monocyte and granulocyte adherence, chemotaxis and migration into inflammatory sites: insights from an experiment of nature. In: Evered D, Nugent J, O'Connor M (eds.). Biochemistry of macrophages (Ciba Symposium 118). Pitman, London, 1986, pp 102–126
5. Martz E: LFA-1 and other accessory molecules functioning in adhesions of T and B lymphocytes. Hum Immunol 18:3–37, 1987
6. Sanchez-Madrid F, Nagy JA, Robbins E, Simon P, Springer TA: Characterization of a human leucocyte differentiation antigen family with distinct α subunits and a common β subunit: the lymphocyte-function associated antigen (LFA-1), the C3bi complement receptor

(OKM1/Mac-1), and the p 150,95 molecule. J Exp Med 158:1785–1803, 1983

7. Springer TA, Thompson WS, Miller J, Schmalstieg FC, Anderson DC: Inherited deficiency of the Mac-1, LFA-1, p 150,95 glycoproteins family and its molecular basis. J Exp Med 160:1901–1918, 1984

8. Sanchez-Madrid F, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff SJ, Springer TA: Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. Proc Natl Acad Sci USA 79:7489–7493, 1982

9. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ: The functional significance, distribution, and structure of LFA-1, LFA-2, and LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol 131:611–616, 1983

10. Mentzer SJ, Gromkowski SH, Krensky AM, Burakoff SJ, Martz E: LFA-1 membrane molecule in the regulation of homotypic adhesions of human B lymphocytes. J Immunol 135:9–11, 1985

11. Haskard D, Cavender D, Beatty P, Springer T, Ziff M: T lymphocyte adhesion to endothelial cells: mechanism demonstrated by anti-LFA-1 monoclonal antibodies. J Immunol 137:2901–2906, 1986

12. Patarroyo M, Beatty PG, Fabre JW, Gahmberg CG: Identification of a cell surface protein complex mediating phorbol ester-induced adhesion (binding) among human mononuclear leukocytes. Scand J Immunol 22:171–182, 1985

13. Mentzer SJ, Burakoff SJ, Faller DV: Adhesion of T lymphocytes to human endothelial cells is regulated by the LFA-1 membrane molecule. J Cell Physiol 126:285–290, 1986

14. Berzins T, Axelsson B, Hammarstrom ML, Hammarstrom S, Perlmann P: Studies on the role of lymphocyte function-associated antigen 1 (LFA-1) in T cell activation. Scand J Immunol 27:7–16, 1988

15. Prieto J, Beatty PG, Clark EA, Patarroyo M: Molecules mediating adhesion of T and B cells, monocytes and granulocytes to vascular endothelial cells. Immunology 63:631–637, 1988

16. Rosen H, Gordon S: Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells in vitro and inflammatory cell recruitment in vivo. J Exp Med 166:1685–1701, 1987

17. Miller LJ, Baiton DF, Borregaard N, Springer TA: Stimulated mobilization of monocyte Mac-1 and p 150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. J Clin Invest 80:535–544, 1987

18. Ho MK, Springer TA: Biosynthesis and assembly of the alpha and beta subunits of Mac-1, a macrophage glycoprotein associated with complement receptor function. J Biol Chem 258:2766–2769, 1983

19. Ferrari C, Sansoni P, Rowden G, Manara GC, Torresani C, De Panfilis G: One half of the CD11b+ human peripheral blood T lymphocytes coexpress the S-100 protein. Clin Exp Immunol 72:357–361, 1988

20. De Panfilis G, Rowden G, Manara GC, Ferrari C, Torresani C, Sansoni P: The S-100β protein in normal human peripheral blood is uniquely present within a discrete suppressor T cell compartment. Cell Immunol 114:398–404, 1988

21. Anderson DC, Miller LJ, Schmalstieg FC, Rothlein R, Springer TA: Contribution of the Mac-1 glycoprotein family to adherence-dependent granulocyte functions: structure-function assessment employing subunit-specific monoclonal antibodies. J Immunol 137:15–27, 1986

22. Keizer GD, Borst J, Visser W, Schwarting R, De Vries JE, Figdor CG: Membrane glycoprotein p 150,95 of human cytotoxic T cell clones is involved in conjugate formation with target cells. J Immunol 138:3130–3136, 1987

23. Schwarting R, Stein H, Wang CY: The monoclonal antibodies α S-HCL 1 (α Leu-14) and α S-HCL 3 (α Leu-M5) allow the diagnosis of hairy cell leukemia. Blood 65:974–983, 1985

24. Hogg N, Takacs L, Palmer DG, Selvedran Y, Allen C: The p 150,95 molecule is a marker of human mononuclear phagocytes: comparison with expression of class II molecules. Eur J Immunol 16:240–248, 1986

25. Beatty PG, Ledbetter JA, Martin PJ, Price TH, Hansen JA: Definition of a common leukocyte cell-surface antigen (Lp95-150) associated with diverse cell-mediated immune functions. J Immunol 131:2913–2918, 1983

26. Beatty PG, Ochs HD, Harlan JM, Price TH, Rosen H, Taylor RF, Hansen JA, Klebanoff SJ: Absence of monoclonal-antibody-defined protein complex in boy with abnormal leukocyte function. Lancet 535–537, 1984

27. Axberg I, Ramstaedt U, Patarroyo M, Beatty P, Wigzell H: Inhibition of natural killer cell cytotoxicity by a monoclonal antibody directed against adhesion-mediating protein gp 90 (CD18). Scand J Immunol 26:547–554, 1987

28. Austyn JM, Morris PJ: T-cell activation by dendritic cells: CD18-dependent clustering is not sufficient for mitogenesis. Immunology 63:537–543, 1988

29. Katz SI, Tamaki K, Sachs DH: Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 282:324–326, 1979

30. Flotte TJ, Murphy GF, Bhan AK: Demonstration of T200 on human Langerhans cell surface membranes. J Invest Dermatol 82:535–537, 1984

31. Romani N, Schuler G, Fritsch P: Ontogeny of Ia-positive and Thy-1-positive leukocytes of murine epidermis. J Invest Dermatol 86:129–133, 1986

32. De Panfilis G: ATPase, antigens detected by monoclonal antibodies, ultrastructural characters: which is the best marker for Langerhans cells? Arch Dermatol Res 275:407–411, 1983

33. De Panfilis G, Manara GC, Ferrari C, Manfredi G, Allegra F: Imbalance in phenotypic expression of T cell subpopulations during different evolutional stages of lichen planus lesions. Acta Dermato-Venereol 63:369–373, 1983

34. De Panfilis G, Manara GC, Ferrari C, Torresani C: Simultaneous colloidal gold immunoelectronmicroscopy labeling of CD1a, HLA-DR and CD4 surface antigens of human epidermal Langerhans cells. J Invest Dermatol 91:547–552, 1988

35. Manara GC, Ferrari C, Scandroglio R, Rocchi G, Pagani L, De Panfilis G: Characterization of two morphologically distinct Leu-7+ cell subsets with respect to Leu-15 antigen. Scand J Immunol: 23:225–231, 1986

36. De Panfilis G, Manara GC, Ferrari C, Torresani C, Sansoni P: Hairy cell leukemia cells express CD1a antigen. Cancer 61:52–57, 1988

37. De Harven E, Soligo D: Scanning electron microscopy of cell surface antigens labeled with colloidal gold. Am J Anat 175:277–287, 1986

38. Manara GC, Soligo D, Lambertenghi-Deliliers G, Ferrari C, De Panfilis G: Immunogold (IG) scanning electron microscopy (SEM) of melanocytes. Pigm Cell Res 1:300(A), 1988

39. Matutes E, Catovsky D: The fine structure of normal lymphocyte subpopulations. A study with monoclonal antibodies and the immunogold technique. Clin Exp Immunol 50:416–425, 1982

40. De Panfilis G, Ferrari C, Manara GC: An in situ immunogold method applied to the identification of plasma membrane-associated antigens of skin-infiltrating cells. J Invest Dermatol 87:510–514, 1986

41. Ferrari C, De Panfilis G, Manara GC: Preembedding immunogold staining of cell surface-associated antigens performed on suspended cells and tissue sections. In: Hayat MA (ed.). Colloidal Gold: principles, methods, and applications, Vol. I, Academic Press, San Diego and London, in press

42. Schmitt D, Faure M, Dambuyant-Dezutter C, Thivolet J: The semi-quantitative distribution of T4 and T6 surface antigens on human Langerhans cells. Br J Dermatol 111:655–661, 1984

43. De Panfilis G, Manara GC, Ferrari C, Torresani C: CD11 expression by Langerhans cells. J Invest Dermatol 89:310(A), 1987

44. De Panfilis G, Manara GC, Ferrari C, Torresani C, Donelli S, Caleffi E: Reactivity of Langerhans cells with anti-CD11c monoclonal antibody. In: Thivolet J, Schmitt D (eds.): The Langerhans Cell, Colloque Inserm/John Libbey Eurotext, London, 1988, pp 149–157

45. De Panfilis G, Manara GC, Ferrari C, Torresani C: Langerhans cells express the adhesion-mediating protein gp95. An immunogold study in electron microscopy. Clin Exp Dermatol (A), in press

46. De Panfilis G, Manara GC, Ferrari C, Soligo D, Torresani C, Zucchi A: Semi-quantitative analysis of the expression of the leukocyte adhesion-promoting heterodimer family (CD11a, b, c/CD18) on Langerhans cells. J Invest Dermatol 91:391(A), 1988

47. Micklem KJ, Law SK, Mason DY: Epitope masking of CD18 antigen. In: McMichael AJ (ed.). Leucocyte Typing III. White cell differentiation antigens. Oxford University Press, Oxford, 1987, pp 834–836

48. Davignon D, Martz E, Reynolds T, Kurzinger K, Springer TA: Monoclonal antibody to a novel lymphocyte function-associated antigen (LFA-1): mechanism of blockade of T lymphocyte-mediate killing and effects on other T and B lymphocyte functions. J Immunol 127:590–595, 1981
49. Springer TA, Davignon D, Ho MK, Kurzinger K, Martz E, Sanchez-Madrid F: LFA-1 and Lyt-2,3, molecules associated with T lymphocyte-mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. Immunol Rev 68:171–195, 1982
50. Gromkowsky SH, Krensky AM, Martz E, Burakoff SJ: Functional distinctions between the LFA-1, LFA-2, and LFA-3 membrane proteins on human CTL are revealed with trypsin-pretreated target cells. J Immunol 134:244–249, 1985
51. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL 1 and interferon-γ: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol 137:245–254, 1986
52. Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137:1270–1274, 1986
53. Harrist TJ, Bhan AK, Murphy GF, Sato S, Berman RS, Gellis SE, Freedman S, Mihm MC Jr: In situ characterization of cutaneous infiltrates with monoclonal antibodies. Am J Clin Pathol 79:294–300, 1983
54. Ralfkiaer E, Stein H, Plesner T, Hou-Jensen K, Mason D: In situ immunological characterization of Langerhans cells with monoclonal antibodies: comparison with other dendritic cells in skin and lymph nodes. Virchows Arch [A] 403:401–412, 1984
55. Hancock WW, Atkins RC: Immunohistologic analysis of the cell surface antigens of human dendritic cells using monoclonal antibodies. Transplant Proc 16:963–967, 1984
56. Bos JD, Sillevis JH, Krieg SR, Bakker PM, Vos GD, Zaane DJ: Acute disseminated histiocytosis-X: *in situ immunophenotyping* with monoclonal antibodies. J Cutan Pathol 11:59–64, 1984
57. Bieber T, Hanau D, Heid E, Kazatchkine MD: Histiocytosis-X cells express C3b, C3d, and C3bi receptor (CR1, CR2, and CR3) antigens. Arch Dermatol Res 277:496–498, 1985
58. Groh V, Tani M, Harrer A, Wolff K, Stingl G: Leu-3/T4 expression on epidermal Langerhans cells in normal and diseased skin. J Invest Dermatol 86:115–120, 1986
59. Stingl G, Katz SI, Abelson LD, Mann DL: Immunofluorescent detection of human B cell alloantigens on S-Ig-positive lymphocytes and epidermal Langerhans cells. J Immunol 120:661–664, 1978
60. Rowden G, Phillips TM, Lewis MG: Ia antigens on indeterminate cells of the epidermis: immunoelectronmicroscopic studies of surface antigens. Br J Dermatol 100:531–542, 1979
61. Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee JO'D: Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab Invest 54:322–335, 1986
62. Dezutter-Dambuyant C, Cordier G, Schmitt D, Faure M, Laquoi C, Thivolet J: Quantitative evaluation of two distinct cell populations expressing HLA-DR antigens in normal human epidermis. Br J Dermatol 111:1–11, 1984
63. Austyn JM: Lymphoid dendritic cells. Immunology 62:161–170, 1987
64. Silberberg I: Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. An ultrastructural study. Acta Derm Venereol 53:1–12, 1973
65. Silberberg I, Baer RL, Rosenthal SA: The role of Langerhans cells in contact allergy. I. An ultrastructural study in actively induced contact dermatitis in guinea pigs. Acta Dermato-Venereol 54:321–331, 1974
66. Silberberg-Sinakin I, Thorbecke J: Contact hypersensitivity and Langerhans cells. J Invest Dermatol 75:61–67, 1980
67. Gothelf Y, Hanau D, Tsur H, Sharon N, Sahar E, Cazenave JP, Gazit E: T6 positive cells in the peripheral blood of burn patients: are they Langerhans cells precursors? J Invest Dermatol 90:142–148, 1988
68. Silberberg-Sinakin I, Thorbecke GJ, Baer RL, Rosenthal SA, Berezowsky V: Antigen-bearing Langerhans cells in skin, dermal lymphatics and in lymph nodes. Cell Immunol 25:137–151, 1976